

APPENDIX A

Please amend claims 1-5, 7-9, 14-18, 22, 28, 37-38, 41-42, 49-51 and 54, to read as follows:

1. A method of formulating a composition comprising one or more chemokines for use in a pharmaceutical composition having anti-HIV activity against one or more HIV-1 isolates present in an individual at a given time, the method comprising:

(a) contacting a first aliquot of HIV⁺ cells obtained from said individual with a chemokine compound, wherein the chemokine compound comprises a member selected from the group consisting of:

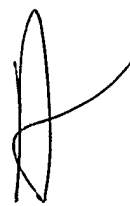
(i) at least one chemokine selected from the group consisting of MDC, MCP-1, MCP-2, MCP-3, MCP-4, MIP-1 γ , MIP-3 α , MIP-3 β , eotaxin, Exodus, I-309, γ IP-10, PF4, NAP-2, GRO- α , GRO- β , GRO- γ , ENA-78, GCP-2, and lymphotactin; and

(ii) at least one chemokine of (i) comprising one or more conservative substitution, terminal additions and/or terminal deletions [derivative and/or chemokine analog]; and

(b) comparing the ability to isolate HIV from said cells with the ability to isolate HIV from a second aliquot of HIV⁺ cells obtained from said individual that are not contacted with said chemokine compound; [s, chemokine derivatives and/or chemokine analogs;]

(c) formulating the composition to comprise the chemokine compound [one or more chemokines, chemokine derivatives and/or chemokine analogs,] which produces a decrease in the ability to isolate virus in the presence of said chemokine compound in the HIV⁺ cells of the individual. [chemokines, chemokine derivatives and/or chemokine analogs.]

2. The method of claim 1, further comprising the step of combining in the composition two or more of said chemokines, [chemokine derivatives and/or chemokine analogs] demonstrating anti-viral activity against said HIV-1 isolates.



3. The method of claim 2 wherein at least 3 of said chemokines[, chemokine derivatives and/or chemokine analogs] are combined.
4. The method of claim 1 further comprising repeating said contacting and comparing steps for at least 2 individual chemokines[, chemokine derivatives and/or chemokine analogs].
5. The method of claim 1 further comprising repeating said contacting and comparing steps for at least 3 individual chemokines[, chemokine derivatives and/or chemokine analogs].
7. The method of claim 1 wherein the HIV⁺ cells are co-cultured with uninfected CD4⁺ peripheral blood mononuclear cells prior to said contacting with the chemokines[, chemokine derivatives and/or chemokine analogs].
8. A method of formulating a pharmaceutical composition for a particular [human] subject infected with HIV, the method comprising:

assaying at least one chemokine[, chemokine derivative and/or chemokine analog] for the ability to inhibit:

HIV infection;

HIV replication; or

expression of an RNA or protein of HIV;

wherein said HIV is a primary isolate recovered from said subject; and

combining an amount effective [for therapy of a disease or disorder associated with HIV infection] of one or more of said chemokines[, chemokine derivatives and/or chemokine analogs] demonstrating said ability with a pharmaceutically acceptable carrier [suitable for use *in vivo* in humans] to decrease viral load in the isolate of said subject.

9. The method of claim 8 [9] wherein said assaying of the chemokine[, derivative, or analog] is by a method comprising:

measuring HIV-1 levels in primary macrophage cells or primary CD4⁺ peripheral blood mononuclear cells incubated with the primary isolate, which cells have been contacted with the chemokine[s, chemokine derivatives and/or chemokine analogs]; and



comparing the measured HIV-1 levels in the cells which have been contacted with the chemokine[s, chemokine derivatives and/or chemokine analogs] with said levels in cells not so contacted with the chemokine[s, chemokine derivatives and/or chemokine analogs], wherein a lower level in said contacted cells indicates that the chemokine[s, chemokine derivatives and/or chemokine analogs have] has anti-HIV activity.

14. The method of claim 8 wherein said assaying of the chemokine[s, chemokine derivatives and/or chemokine analogs] is by a method comprising:

measuring HIV-1 levels in cultures of HIV⁺ cells obtained from the [patient] subject which have been contacted with the chemokine[s, chemokine derivatives and/or chemokine analogs]; and

comparing said measured HIV-1 levels with said levels in said cells not so contacted with the chemokine[s, chemokine derivatives and/or chemokine analogs], wherein a lower HIV-1 level in cultures of said contacted cells indicates that the chemokine[s, chemokine derivatives and/or chemokine analogs] has anti-HIV activity.

15. The method of claim 14 further comprising repeating steps (a) and (b) for at least 2 individual chemokines[, or derivatives or analogs].

16. The method of claim 8[14] wherein the chemokine is a chemokine derivative and/or chemokine analog [further comprising repeating steps (a) and (b) for at least 3 individual chemokines, or derivatives or analogs].

17. The method of claim 15 or 16 wherein the chemokine[s, derivatives, or analogs are] is selected from the group consisting of MDC, MCP-1, MCP-2, MCP-3, MCP-4, MIP-1 γ , MIP-3 α , MIP-3 β , eotaxin, Exodus, I-309, γ IP-10, PF4, NAP-2, GRO- α , GRO- β , GRO- γ , ENA-78, GCP-2, and lymphotactin.

18. A method of inhibiting [treating or preventing] HIV infection or replication in cells of a [human] subject in need of such treatment, the method comprising administering to the subject a pharmaceutical composition comprising:

at least one [a] chemokine selected from the group consisting of MDC, MCP-1, MCP-2, MCP-3, MCP-4, MIP-1 γ , MIP-3 α , MIP-3 β , eotaxin, Exodus, I-309, γ IP-10, PF4, NAP-2, GRO- α , GRO- β , GRO- γ , ENA-78, GCP-2, and lymphotactin in an amount effective to inhibit HIV infection or replication in the cells of the subject; and

a pharmaceutically acceptable carrier.

22. A method of decreasing a HIV viral load [treating or preventing of HIV infection or replication] in a [human] subject in need of such treatment, the method comprising administering to the subject a pharmaceutical composition comprising:

at least one [a] nucleic acid encoding a chemokine selected from the group consisting of MDC, MCP-1, MCP-2, MCP-3, MCP-4, MIP-1 γ , MIP-3 α , MIP-3 β , eotaxin, Exodus, I-309, γ IP-10, PF4, NAP-2, GRO- α , GRO- β , GRO- γ , ENA-78, GCP-2, and lymphotactin, in an amount effective to decrease HIV viral load [inhibit HIV infection or replication]; and

a pharmaceutically acceptable carrier.

28. The method of claim [25] 22 further comprising administering to the subject an anti-viral drug other than a chemokine, in an amount effective to inhibit HIV infection or replication.

37. A method of inhibiting [treating or preventing] HIV infection or replication in a [human] subject in need of such treatment, the method comprising administering to the subject a composition comprising:

a first nucleic acid encoding RANTES, MIP-1 α , MIP-1 β , or IL-8, and

a second nucleic acid encoding a chemokine selected from the group consisting of MCP-1, MCP-2, MCP-3, MCP-4, MIP-1 γ , MIP-3 α , MIP-3 β , eotaxin, Exodus, I-309, γ IP-10, PF4, NAP-2, GRO- α , GRO- β , GRO- γ , ENA-78, GCP-2, lymphotactin and SDF-1;

together in an amount effective to inhibit HIV infection or replication.

38. A pharmaceutical composition comprising:

at least one [a] chemokine selected from the group consisting of MDC, MCP-2, MCP-4, MIP-1 γ , MIP-3 α , MIP-3 β , eotaxin, Exodus, I-309, γ IP-10, PF4, NAP-2, GRO- α , GRO- β , GRO- γ , ENA-78, GCP-2, and lymphotactin, in an amount effective to decrease a HIV viral load in infected cells [inhibit HIV infection or replication]; and

a pharmaceutically acceptable carrier.

41. The pharmaceutical composition of claim 38 further comprising at least one member selected from the group consisting of RANTES, MIP-1 α , MIP-1 β , MCP-1, MCP-3, IL-8 or SDF-1 together in an amount effective to inhibit HIV infection or replication.
42. The pharmaceutical composition of claim 41 wherein the chemokine is a chemokine derivative and/or chemokine analog [s are purified].
49. A pharmaceutical composition comprising:
- two or more chemokines, each of which binds to at least one chemokine receptor selected from the group consisting of CC CKR-1, CC CKR-2A, CC CKR-2B, CC CKR-3, CC CKR-4, CC CKR-5, CxC CKR4, IL-8RA, IL-8RB, Mig receptor, γ IP-10 receptor and Duffy antigen, in an amount effective to inhibit HIV infection or replication in infected cells; and
- a pharmaceutically acceptable carrier.
50. A method of formulating a pharmaceutical composition having anti-HIV activity against one or more HIV-1 isolates present in an individual at a given time, the method comprising:
- contacting a first aliquot of CD4⁺ cells, one or more virus isolates obtained from said individual, and a chemokine[, chemokine derivative and/or chemokine analog]; and
- comparing the ability to isolate HIV from said cells with the ability to isolate HIV from a second aliquot of CD4⁺ cells contacted with said virus isolates that are not contacted with said chemokines[, chemokine derivatives and/or chemokine analogs],
- wherein a decrease in the ability to isolate virus in the presence of said chemokines[, chemokine derivatives and/or chemokine analogs] is indicative that the chemokines[, chemokine derivatives and/or chemokine analogs] has anti-viral activity against said HIV-1 isolates.
51. A pharmaceutical composition comprising MDC and I-309 in an effective amount to exhibit anti-HIV activity in human cells; and a pharmaceutically acceptable carrier.
54. The method of claim [52] 18 wherein the MDC and I-309 are administered in a synergistically effective and therapeutically effective amount.